A Dual-Chamber Chemostat for the Study of Algal Interactions

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November 1986

Canadian Technical Report of Fisheries and Aquatic Sciences No. 1488

Fisheries and Oceans Pêches et Océans

Canada

87-02092/

Canadian Technical Report of Fisheries and Aquatic Sciences

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from the Central and Arctic Region, Winnipeg

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Cat. no. Fs 97-6/1488E ISSN 0706-6457

Correct citation for this publication is:

Hendzel, L.L. 1986. A dual-chamber chemostat for the study of algal interactions. Can. Tech. Rep. Fish. Aquat. Sci. 1488: iv + 7 p.

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ABSTRACT

Hendzel, L.L. 1986. A dual-chambered chemostat for the study of algal interactions. Can. Tech. Rep. Fish. Aquat. Sci. 1488: iv + 7 p.

A two-chambered chemostat to study competitive growth interactions in two distinct algal populations is described. Medium inflow and culture outflow was provided by a peristaltic pump while equalized air pressure within the chemostat maintained equal volumes of culture on either side of the membrane.

Growth parameters of two species of algae (Synechococcus linearis and Scenedesmus quadricauda) in the dual-chamber chemostat were comparable to those measured for the same species in individual chemostats. Growth rates, biomass estimators and physiological ratios were similar between the two types of chemostats suggesting the apparatus introduced no additional growth perterbations. Reduced yield of S. linearis in the dual-chamber chemostat may indicate an effect due to S. quadricauda.

Key words: two-chambered chemostat; competition; porous membrane; deficiency; growth parameters.

RÉSUMÉ

Hendzel, L.L. 1986. A dual-chambered chemostat for the study of algal interactions. Can. Tech. Rep. Fish. Aquat. Sci. 1488: iv + 7 p.

L'auteur décrit un chemostat à deux chambres permettant d'étudier la compétition au cours de la croissance de deux populations d'algues. L'apport de milieux et la décharge de cultures s'effectuent à l'aide d'une pompe péristaltique tandis qu'une pression atmosphérique équilibrée à l'intérieur du chemostat maintient un volume égal de cultures des deux côtés de la membrane.

Les paramètres de croissance de deux espèces d'algue (Synechococcus linearis et Scenedesmus quadricauda) obtenus à l'aide de cet appareil étaient semblables à ceux mesurés chez les mêmes espèces dans des chemostats individuels. Les taux de croissance, les estimateurs de la biomasse et les rapports physiologiques étaient pareils, ce qui porte à croire que le chemostat à deux chambres n'apporte aucune perturbation supplémentaire de la croissance. Un rendement plus bas de S. linearis dans cet appareil peut indiquer un effet de S. quadricauda.

Mots-clés: chemostat à deux chambres; compétition; membrane poreuse; insuffisance; paramètres de croissance.

INTRODUCTION

Novick and Szilard (1950) first described a chemostat for the continuous culturing of bacteria under nutrient limitation. Since then the theory of chemostat operation has been elucidated by Herbert (1958), Herbert et al. (1956), and more recently by Tempest (1970). Today the chemostat is an accepted tool for culturing micro-organisms for physiological experiments. Similar to the classical chemostat, are "cage" or dialysis cultures (Sakshaug and Jensen 1978) where the organisms can be maintained under constant conditions of nutrient supply within or behind a permeable membrane which allows for the transport of low molecular weight dissolved components of the liquid medium, including soluble metabolites and soluble pollutants, into and out of the culture. With this technique, a specific organism can be maintained in a discrete enclosure but still be in contact with the environment in question. These cage cultures have included flow-through designs with permeable membranes (Skipnes et al. 1980) and disposable hollow dialysis fiber units (hemodialyzer) attached to a culture vessel (Marsot et al. 1981).

In the study of growth interactions amongst algae, Tilman and Kilham (1976) used simple batch culture and semi-continuous cultures to describe competition between two freshwater diatoms for phosphate and silicate. Kroes (1971, 1973) used more complex filter-culture vessels, where culture media was actively exchanged between two algal cultures, separated by filters. However, both approaches lack versatility and limit investigations to cell counts and observations of morphological changes. Any growth related experiments on the separate component species grown in a shared medium at specific growth rates are unattainable or at best difficult with these approaches. These shortcomings led to the development of a dual-chamber chemostat where two species of algae can be maintained indefinitely at steady-state, growing in a similar, shared environment but physically separated by a porous membrane.

METHODS-APPARATUS

The dual-chamber chemostat (Fig. 1) consists of two Pyrex reaction kettles (11 cm O.D. x 17 cm L). The ground glass flanges sandwich two 142 mm Teflon-coated filter support screens (Millipore YY40142-64, Millipore Ltd., Mississauga, Ont.) which in turn hold a 142 mm polycarbonate filter (Nuclepore #112109, Nuclepore Corp., Pleasanton, CA 94566) with a pore diameter of 0.8 µM. All mating surfaces were lightly greased with Dow-Corning High Vacuum silicone grease, and both vessel halves, filter supports and filter were held together with the appropriate kettle clamp. Each chamber was fitted with a 25 mm I.D. x 30 mm H glass opening, which accepted a #4 silicone stopper. Through each stopper passed four 2 mm I.D. glass tubes. Liquid medium and air were introduced together through a special fitting (see insert Fig. 1) which allowed both to pass down the same tube to the bottom of the chemostat. A second glass tube of equal length was connected by silicone tubing to a sampling flask. The third glass tube of short length allowed for the controlled release of air. The fourth glass tube, equal in length to the first two, allowed culture to be pumped out of each side of the chemostat at a rate equal to that of the incoming medium. The standpipe overflow type of system to remove excess culture could not be used because reciprocating shaking was used to provide mixing in the culture chambers. A Harvard peristaltic pump (Harvard Apparatus Company, Inc., Millis, Massachusetts 02054) provided constant medium addition and culture removal. It was found early in the development of the dual-chamber chemostat that aeration of each chamber could produce volume changes between the two sides; that is, medium could be driven from one chamber to accumulate in the other due to differences in incoming air pressure and volume. Careful balancing of the incoming air helped but did not cure the problem; however, control of the effluent air did. By interconnecting and directing the effluent air from each chamber to a 1m long glass tube standpipe set in a larger diameter tube of similar length filled with water (Fig. 1) equal backpressure could be maintained in each chamber. The exhausting of effluent air was controlled by a release valve and was set so that the standpipe displaced downward approximately 15 cm of water. The desired amount of backpressure in the system could then be controlled by adjustment of the air release valve.

Using aseptic technique, each chamber was inoculated by injecting approximately 5 mL of inolculum from a syringe fitted with a 15 cm L x 18 gauge spinal needle through the centre of the medium/air fitting before connecting the incoming medium tubing. The dual-chamber chemostat, once inoculated and connected to the medium reservoirs (8 L aspirator bottles) (Fig. 2), was placed in a plexiglass cradle and fastened to the top of a reciprocal shaker (New Brunswick Scientific Co., Inc., New Brunswick, New Jersey 08903). Growth experiments were conducted in a controlled temperature room at 20°C. Constant illumination from one side was provided by a bank of 20 W vita-lite fluorescent tubes (Duro-Test Electric Ltd., Rexdale, Ont.) providing 60 $\mu E \cdot m^{-2} \cdot s^{-1}$ at the chemostat surface. The reciprocal shaker, set at 120 strokes per minute (spm), provided a moderate back and forth rocking motion to the cultures. When a volume of each culture was removed for sampling, an equal volume of medium was immediately introduced into each chamber by increasing the flow rate for a short period; otherwise the medium/effluent pump was run at a set speed so as to provide the required dilution rates.

DISCUSSION

To estimate the rate of exchange across the membrane filter, the dual-chamber chemostat was assembled with a 0.8 µM pore size Nuclepore filter in place. A solution of 500 mL of 1.0 μMol O-methylfluorscein$\cdot L^{-1}$ in 0.05 N NaOH was added to the left chamber (chamber A) of the chemostat while an equal volume of 0.05 N NaOH was added to the right chamber (chamber B). Over the course of seven hours, while being

shaken at 120 spm, the disappearance of fluorescence in chamber A (Fig. 3) and its appearance in chamber B were monitored using a fluorometer (Turner model #111, Turner Instrument Co., Ltd.). While equilibrium was not reached during the period, the diffusion rate was approximately half the concentration in 2 h. Larger organisms would allow the use of a filter with a larger pore size resulting in shorter equilibrium times. These results indicate that, except for very labile compounds, any dissolved metabolite produced in one chamber should approach similar concentrations in both chambers during steady-state operation of the chemostat.

The growth characteristics of two species of algae growing in the dual-chamber chemostat were compared with those of the same species growing in two individual chemostats, at three dilution rates, 0.07, 0.14 and 0.28 day^{-1}. One of the individual chemostats and one chamber of the dual-chamber chemostat were inoculated with Scenedesmus quadricauda (Turp) deBreb., the others with Synechococcus linearis (Nag.) Kom (Cultures 11 and 27 respectively in the Freshwater Institute culture collection). Both chemostat and inoculum cultures were grown in medium WC (Guillard and Lorenzen 1972), with K_2HPO_4 being replaced with equimolar KCl and KH_2PO_4 added to a final concentration of 2 μMol $P \cdot L^{-1}$. All chemostats had a volume of 700 mL. Growth was measured as the increase of optical density of each individual culture as measured on a Spectronic 100 spectrophotometer (Bauch and Lomb) (Fig. 4). Growth rate, expressed as doublings per day (k_2), was similar for all four cultures during exponential growth, ranging from 1.14 for S. linearis in the dual-chamber chemostat to 1.25 for the same alga in the individual chemostat. Based on the exponential doubling rates, it appears that growth in the dual-chamber chemostat had no adverse effect on growth rates when compared with the individual chemostats; however, the reduced yield of S. linearis in the dual-chamber chemostat when compared with the same alga in the individual chemostat may indicate an effect of S. quadricauda on S. linearis mediated through some dissolved metabolite produced by the former.

Figure 5 shows a number of growth parameters which illustrate other similarities and differences between the dual-chamber and single-chamber chemostats. Estimators of biomass (cell numbers and particulate carbon) and the physiological ratios (P/C, chlorophyll a/C and P uptake/C) were similar for the two types of chemostats for each of the species. The discrepancies that do show up when comparing these parameters for the two types of chemostats may be due to the fact that each dilution rate represents only one sample. Growth in a shared medium did not change P uptake and yield characteristics relative to growth in separate cultures for these two algae.

CONCLUSION

Where dissolved metabolites have no effect, the outcome of competition between pairs of algae for nutrients can be understood by studying the growth or nutrient uptake and yield characteristics of the individual species in separate cultures (Tilman and Kilham 1976). If one species produces a dissolved metabolite which affects these characteristics in another alga, it could alter the outcome of competition when the two algae are grown together, but not be apparent when the two species are grown separately. By using the dual-chamber chemostat, two algae can be grown in a shared medium, but kept separate so parameters potentially important in competition can be measured in the presence of dissolved metabolites from both species. Thus, the influence of all but the most labile metabolites should be detected.

ACKNOWLEDGEMENTS

I would like to thank Pat Healey for his comments on this manuscript, Mike Pidlubney for drawing the figures and Donna Laroque for typing the manuscript.

REFERENCES

GUILLARD, R.R.L., and C.J. LORENZEN. 1972. Yellow-green algae with chlorophyllide c. J. Phycol. 8: 10-14.

HERBERT, D. 1958. Some principles of continous culture. 7th Int. Congr. Microbiol. 6: 381-396.

HERBERT, D., R. ELSWORTH, and R.C. TELLING. 1956. The continuous culture of bacteria, a theoretical and experimental study. J. Gen. Microbiol. 14: 601-622.

KROES, H.W. 1971. Growth interactions between Danydomonas globosa Snow and Chlorococcum ellipsoideum Deason and Bold under different experimental conditions, with special attention to the role of pH. Limnol. Oceanogr. 16: 869-879.

KROES, H.W. 1973. A spin filter system for the study of algal interactions. Oecologia (Berl.) 11: 93-98.

MARSOT, P., R. FOURNIER, and C. BLAIS. 1981. Cultures à dialyse: emploi de fibres creuses dialysantes pour la culture massive de phytoplancton. Can. J. Fish. Aquat. Sci. 38: 905-911.

NOVICK, A., and L. SZILARD. 1950. Experiments with the chemostat on spontaneous mutations of bacteria. Proc. Natl. Acad. Sci. Wash. 36: 708-719.

SAKSHAUG, E., and A. JENSEN. 1978. The use of cage cultures in the studies of the biochemistry and ecology of marine phytoplankton. Oceanogr. Mar. Biol. Annu. Rev. 16: 81-106.

SKIPNES, O., I. EIDE, and A. JENSEN. 1980. Cage culture turbidostat: a device for rapid determination of algal growth rate. Appl. Environ. Microbiol. 40: 318-325.

TEMPEST, D.W. 1970. The continous cultivation of micro-organisms. 1. Theory of the chemostat, p. 259-276. In J.R. Novis and D.W. Ribbons (ed.) Methods in Microbiology 2. Academic Press, New York.

TILMAN, D., and S.S. KILHAM. 1976. Phosphate and silicate growth and uptake kinetics of the diatom Asterionella formosa and Cyclotella meneghineana in batch and semi-continous culture. J. Phycol. 12: 375-383.

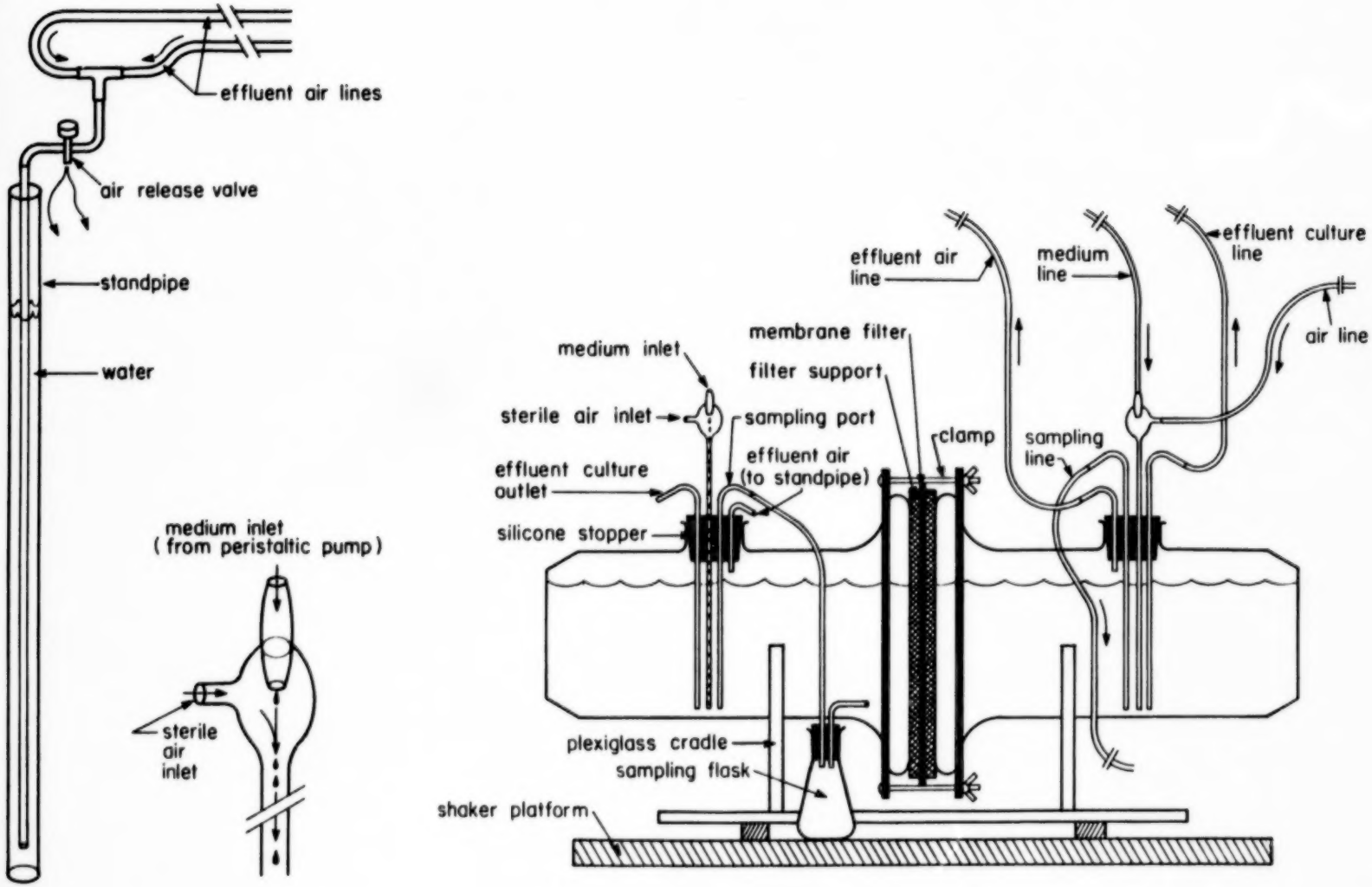

Figure 1. Schematic drawing of dual-chamber chemostat vessel, including standpipe and medium air inlet.

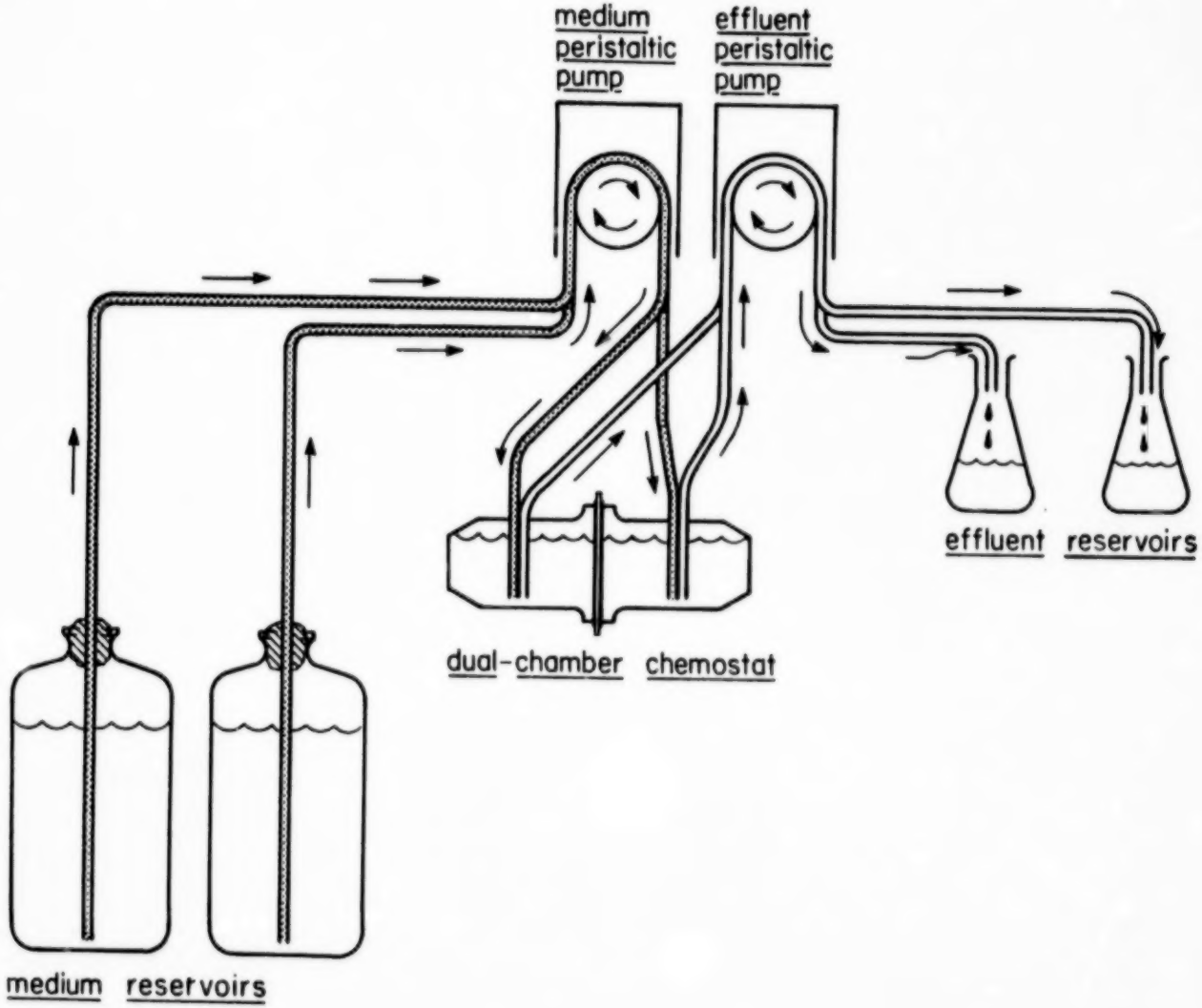

Figure 2. Flow diagram of dual-chamber chemostat.

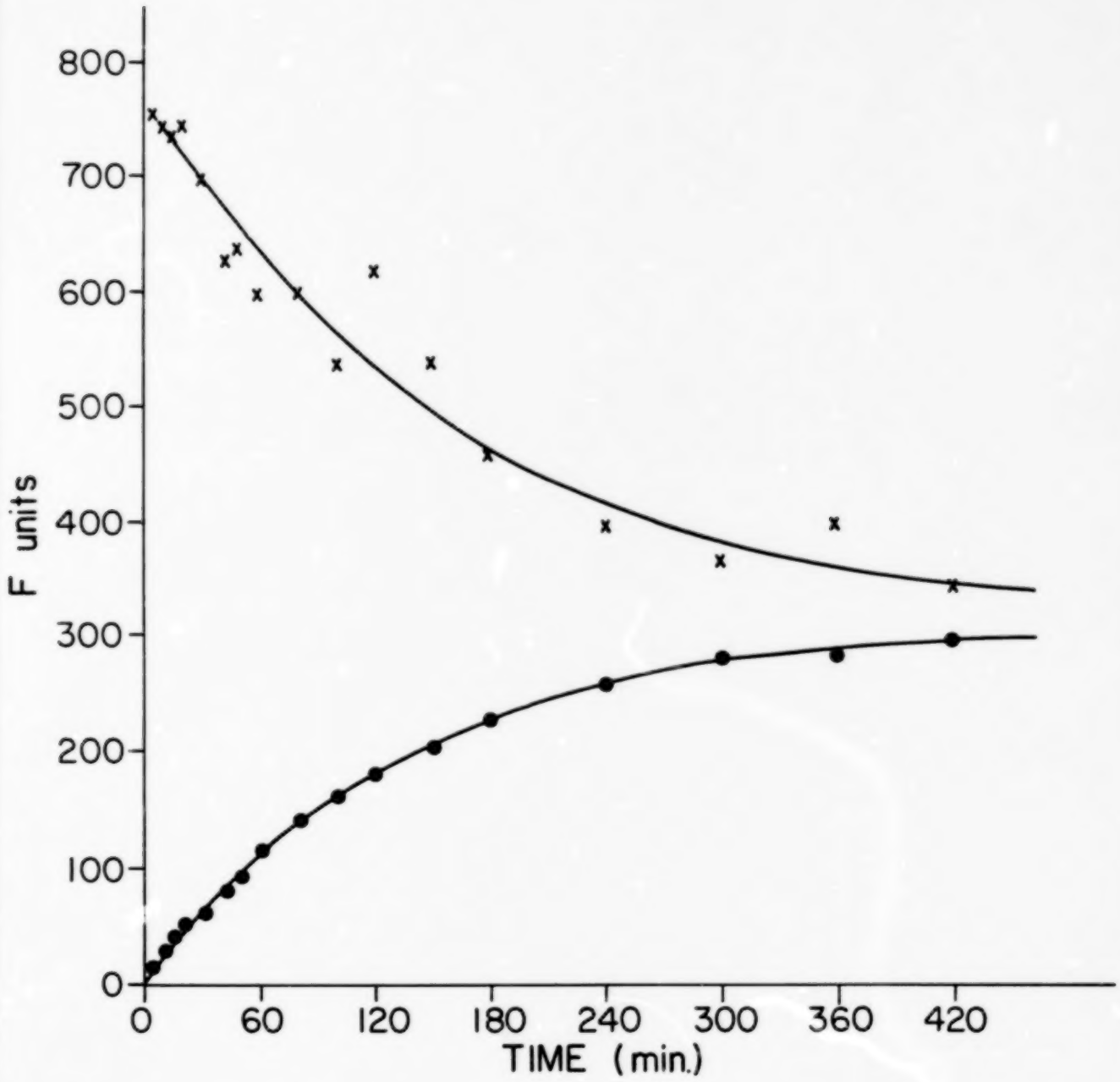

Figure 3. Diffusion rate across the membrane filter.

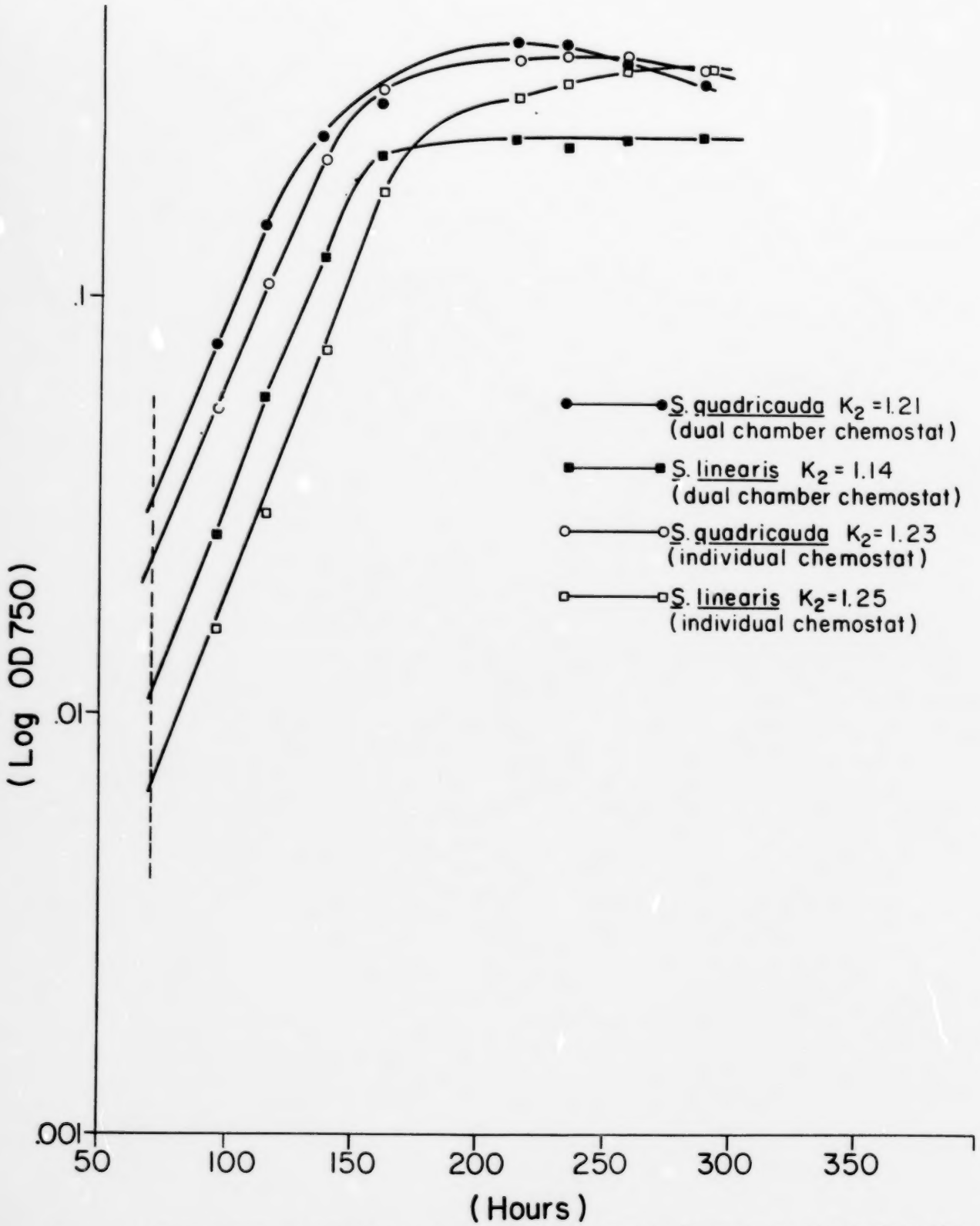

Figure 4. Growth into steady-state of S. quadricauda and S. linearis in the dual-chamber chemostat and in individual chemostats at 20°C and the dilution rate (D) = 0.14.

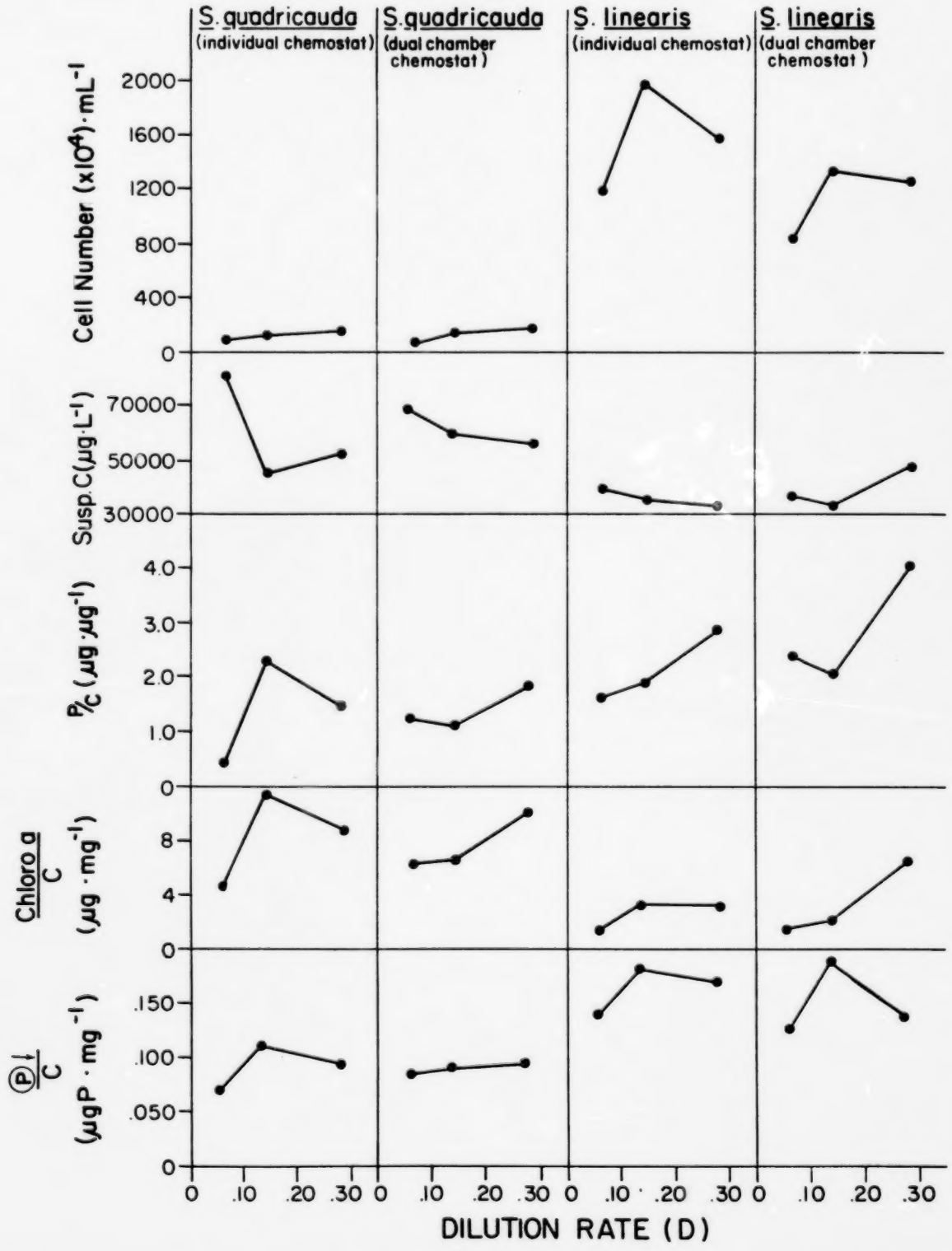

Figure 5. Comparison of growth parameters.